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June 9, 2010

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SUPERFUND DIVISION

Ms. Diana Engeman
Remedial Project Manager
Superfund Division
U.S. Environmental Protection Agency, Region VII
901 North 5th Street
Kansas City, KS 66101

MWH #1007802.0101

RE: Work Plan – Stable Isotope Probing Bio-Trap® Study using EAS™
Former Peoples Natural Gas Site
Dubuque, Iowa

Dear Ms. Engeman:

On behalf of MidAmerican Energy Company (MidAmerican), MWH has prepared this Work Plan for the former Peoples Natural Gas (PNG) site located at 925 Kerper Boulevard in Dubuque, Iowa. Pending United States Environmental Protection Agency (USEPA) approval, it is anticipated this work will be initiated during the summer of 2010 in accordance with the schedule presented in this Work Plan.

Purpose

As requested by USEPA, MidAmerican has evaluated a number of remedial options to address groundwater impact in the vicinity of monitoring well P-112. The purpose of the activities proposed in this Work Plan is to evaluate whether addition of an electron acceptor to the silty sand aquifer at the former PNG site will enhance biodegradation of site contaminants of concern (COCs) consisting of benzene, toluene, ethylbenzene, xylenes (BTEX) and polycyclic aromatic hydrocarbons (PAHs).

Background

A petition for approval of a Technical Impracticability (TI) Waiver for the PNG site was submitted to the USEPA on December 1, 2006 and is currently pending USEPA approval. An August 2009 USEPA technical review of the PNG site indicated a TI Waiver may be appropriate; however, active measures may be warranted to prevent plume migration. Because of a change in local groundwater use, groundwater flow in the silty sand aquifer has changed direction, with current flow toward the Mississippi River. The past and current typical ranges of predominant groundwater flow directions are illustrated in Figure 1. The change in groundwater flow direction has resulted in increased COC concentrations in monitoring well P-112 (Table 1 and Figure 2) located near the Mississippi River.

Addition of an electron acceptor as a barrier application in the vicinity of monitoring well P-112 may be proposed as a means to enhance biodegradation of site COCs, thus decreasing COC concentrations upgradient of the Mississippi River. Low oxygen and nitrate concentrations, and elevated dissolved iron and sulfate concentrations suggest current iron reducing conditions are the predominant terminal electron accepting process in the vicinity of monitoring well P-112 (Jurgens, 2009).

Oxygen is the thermodynamically preferred electron acceptor by microbial populations (aerobic respiration); after oxygen is depleted, nitrate generally becomes the preferred electron acceptor (denitrification) (Wiedemeier, 1999). Due to the higher energy derived from the reactions, both aerobic respiration and denitrification are generally preferred by microbial populations over the iron reduction, assumed to be currently occurring in the vicinity of monitoring well P-112. However, the elevated dissolved iron concentration observed at monitoring well P-112 significantly increases both the oxygen and nitrate demand, reducing the feasibility of using oxygen or nitrate to enhance COC biodegradation. In addition, reaction of oxygen or nitrate with dissolved iron would likely result in precipitation of iron oxyhydroxide compounds (Eckert, 2002; Vance, 2008), risking a long-term reduction in aquifer permeability. The potential loss of aquifer permeability was previously discussed with USEPA and identified as a concern.

Sulfate generally becomes the microbially preferred electron acceptor after oxygen, nitrate, and iron III have been depleted. Iron reducing conditions presumed present in the vicinity of monitoring well P-112 suggest iron III has not been depleted, and elevated sulfate concentrations suggest the absence of wide-spread sulfate-reducing conditions. However, iron reduction and sulfate reduction may occur simultaneously under conditions not limited by the presence of an electron donor (Chapelle, 2009). The contaminants of concern in the P-112 area should act as electron donors; therefore, electron donors are not anticipated to be a limiting factor. In addition, although sulfate reduction results in formation of magnesium carbonate and iron sulfate (Foght, 2008), formation of metal precipitates is expected to be significantly less than with addition of oxygen or nitrate. Because it does not involve the limitations associated with oxygen and nitrogen addition, sulfate addition may be a more favorable alternative for enhanced COC biodegradation. Given the uncertainty of establishing sulfate reducing conditions at the PNG site, a Stable Isotope Probing (SIP) Bio-Trap® study is proposed before proceeding with a full pilot study to investigate whether significantly increasing available sulfate will result in stimulation of sulfate-reducing bacterial populations.

Description of Work

The SIP Bio-Trap® study will employ test and control Bio-Trap® units from Microbial Insights, Inc. (Microbial Insights) of Rockford, Tennessee to evaluate sulfate-enhanced microbial degradation of both benzene and naphthalene in monitoring well P-112. Both units will contain beads having a large surface area for microbial colonization, baited with known quantities of either ^{13}C-labeled benzene or naphthalene. The test unit will be loaded with Electron Acceptor Solution [EAS™], a commercially available sulfate amendment from EOS Remediation, LLC, while the control unit will contain no amendments to represent current aquifer conditions. The ends of each Bio-Trap® unit will be capped with a baffle to isolate the unit in the well screen. A 2.2-foot well screen in monitoring well P-112 limits concurrent Bio-Trap® installation to two units; therefore, benzene and naphthalene will be evaluated sequentially. Descriptions of the Bio-Trap® samplers and EAS™ are provided in Attachments A and B, respectively.

Prior to deployment, monitoring well P-112 will be purged until parameter (temperature, pH, specific conductance, oxidation reduction potential, dissolved oxygen, and turbidity) stabilization is achieved. The ^{13}C-labeled benzene Bio-Traps® will then be deployed within the screened interval of monitoring well P-112. The Bio-Traps® will remain in place for approximately 45 days, after which they will be retrieved. After retrieval of the ^{13}C-labeled benzene Bio-Traps®, monitoring well P-112 will again be purged until parameter stabilization is achieved. The ^{13}C-labeled naphthalene Bio-Traps® will be deployed within the screened interval of monitoring well P-112 and retrieved after approximately 45 days.

After retrieval, the Bio-Traps® will be shipped on ice to Microbial Insights for analysis. Analyses will consist of the following:

- ^{13}C-labeled benzene or naphthalene. Comparison of pre- and post-deployment ^{13}C-labeled benzene and naphthalene provides an estimate of degradation rate. Comparison of the loss of ^{13}C-labeled benzene or naphthalene between the control and EAS™-containing Bio-Trap® units will indicate whether significantly increasing available sulfate will result in stimulation of sulfate-reducing bacterial populations.

- ^{13}C-enriched phospholipid fatty acids (PLFA). Quantification of ^{13}C-enriched PLFA provides an indication of ^{13}C uptake in the microbial biomass, a definitive indicator of microbial degradation.

- ^{13}C-enriched dissolved inorganic carbon. Quantification of ^{13}C-enriched dissolved inorganic carbon provides indication of contaminant mineralization, a definitive indicator of microbial degradation.

- Anions. The analysis will allow comparison of sulfate concentration between the control and EAS™-containing Bio-Trap® units.

- Benzyl succinate synthase gene. The benzyl succinate synthase gene is involved in toluene and xylene degradation.

- Iron reducing/sulfate reducing bacteria. Although analysis cannot differentiate between iron-reducing and sulfate-reducing bacteria, the analysis will provide a general indication of their presence.

Next Steps

If Bio-Trap® results suggest addition of sulfate will significantly enhance biodegradation of site COCs, MidAmerican expects to propose a pilot study of EAS™ injection in the vicinity of monitoring well P-112. A separate pilot study work plan would be developed for USEPA review if favorable results are obtained from the Bio-Trap® study. If the EAS™ amendment does not appear favorable, MidAmerican will continue to evaluate the remedial options, including use of nitrate amendment or oxygen addition.

PROJECT SCHEDULE

The work is anticipated to be completed in accordance with the following schedule:

Milestone	**Approximate Time after USEPA Approval of Work Plan**
Deployment of ^{13}C-labeled benzene Bio-Traps®.	2 Weeks
Retrieval of ^{13}C-labeled benzene Bio-Traps® and deployment of ^{13}C-labeled naphthalene Bio-Traps®.	9 Weeks
Retrieval of ^{13}C-labeled naphthalene Bio-Traps®.	16 Weeks
Summary report to USEPA.	26 Weeks

If you have any questions regarding the site, please contact Kevin Dodson of MidAmerican at (515) 281-2692 or me at (515) 253-0830.

Sincerely,

Kevin G. Armstrong

Kevin G. Armstrong, C.P.G.
Project Manager

/kal:kga
Enclosures
- Table 1 – P-112 Groundwater Monitoring Results
- Figure 1 – Predominant Groundwater Flow Directions in the Silty Sand Aquifer
- Figure 2 – P-112 Concentrations
- Attachment A – Bio-Trap® Sampler Information
- Attachment B -- Electron Acceptor Solution (EAS™) Information

cc: Kevin Dodson, MidAmerican Energy Company
Dan Cook, Iowa Department of Natural Resources
Jim Rost, Iowa Department of Transportation
Barry Lindahl, City of Dubuque
Don Vogt, City of Dubuque

References:

Chapelle, Francis H., Paul M. Bradley, Mary Ann Thomas, and Peter B. McMahon, 2009. Distinguishing Iron-Reducing from Sulfate-Reducing Conditions. Ground Water. 2009.

Eckert, Paul and C.A.J. Appelo, 2002. Hydrogeochemical modeling of enhanced benzene, toluene, ethylbenzene, xylene (BTEX) remediation with nitrate. Water Resources Research. 2002.

Foght, J., 2008. Anaerobic Biodegradation of Aromatic Hydrocarbons: Pathways and Prospects. Journal of Molecular Microbiology and Biotechnology. 2008.

Jurgens, Bryant C., Peter B. McMahon, Francis H. Chapelle, and Sandra M. Eberts, 2009. An Excel® Workbook for Identifying Redox Processes in Ground Water. U.S. Geological Survey. 2009.

Vance, David B., 2008. Iron – The Environmental Impact of a Universal Element. 2 The 4 Technology Solutions. http://2the4.nte/iron.htm. 2008.

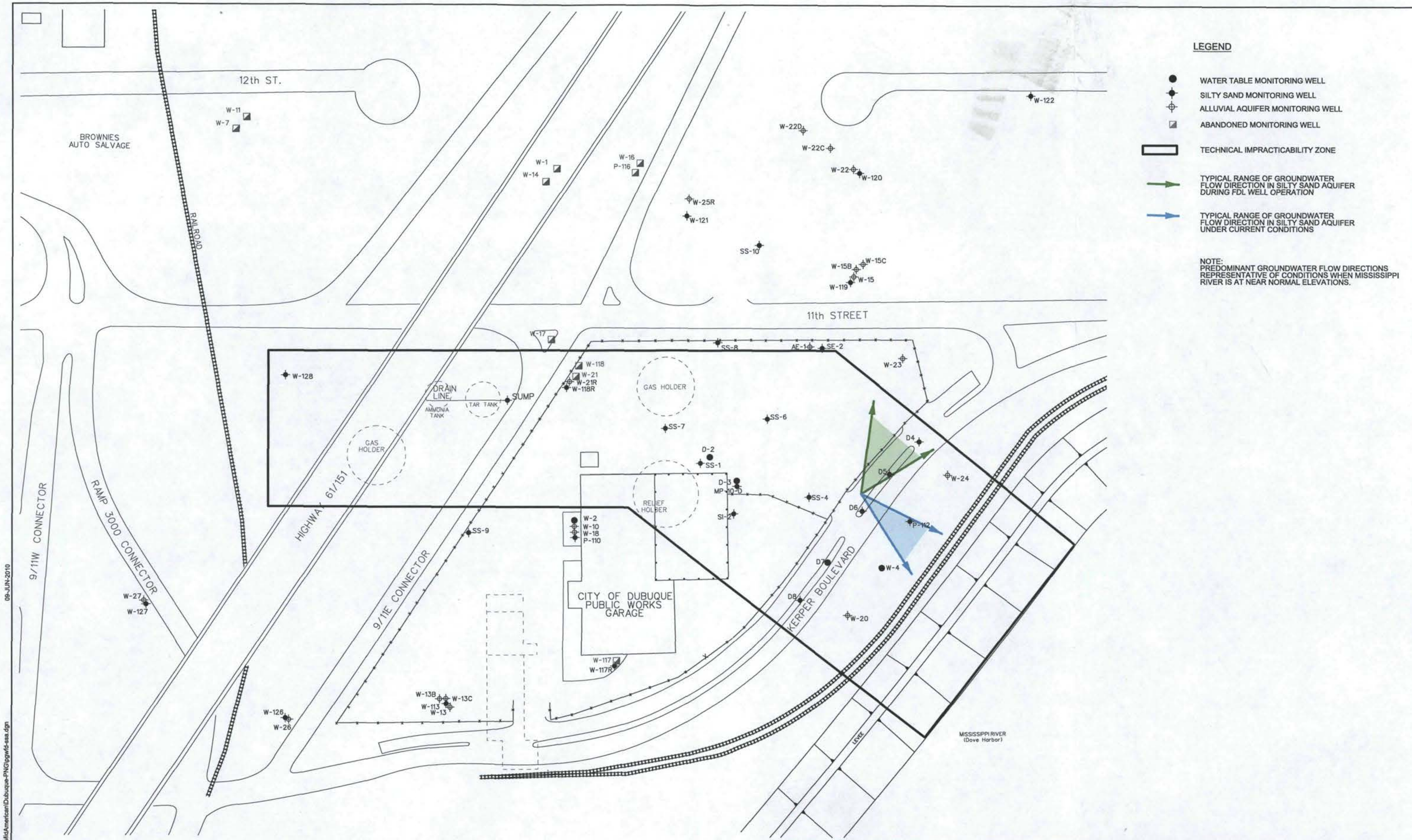
Wiedemeier, T.H., J.T. Wilson, D.H. Kampbell, R.N. Miller, and J.E. Hansen, 1999. Technical Protocol for Implementing Intrinsic Remediation with Long-Term Monitoring for Natural Attenuation of Fuel Contamination Dissolved in Groundwater, vol. 1. Air Force Center for Environmental Excellence, Brooks Air Force Base. March 1999.

TABLE 1
GROUNDWATER ANALYTICAL RESULTS
MIDAMERICAN ENERGY COMPANY
PEOPLES NATURAL GAS SITE
DUBUQUE, IOWA

		Sample Location:	P-112	P-112	P-112	P-112	P-112	P-112	P-112	P-112	P-112	P-112	P-112	P-112
		Screened Unit:	Silty Sand	Silty Sand	Silty Sand	Silty Sand	Silty Sand	Silty Sand	Silty Sand	Silty Sand	Silty Sand	Silty Sand	Silty Sand	Silty Sand
		Depth BTOC (feet):	38.8	38.8	38.8	38.8	38.8	38.8	38.8	38.8	38.8	38.8	38.8	38.8
		Sample Date:	25-Apr-05	11-Oct-05	15-Mar-06	12-Sep-06	18-Apr-07	20-Sep-07	06-May-08	01-Oct-08	29-Apr-09	16-Sep-09	31-Mar-10	06-May-10
Analyte	**Units**	**Remediation Goal**												
Alkalinity, Total as CaCO3	mg/L	--	510	670	810	854	995	1090	1220	1200	1340	1290	1880	na
Ammonia(NH3+NH4),as N	mg/L	--	31	32	32	41.6	41.3	54.3	58.2	74.2	95.7	114	128	na
Chloride	mg/L	--	na	na	na	964 M1	na	na	na	na	na	na	na	na
Iron, Total	mg/L	--	na	95	120	122 MHA	137	101 MHA	65	94.7	78.8	68.8	64.6	na
Iron, Dissolved	mg/L	--	na	99	120	119 MHA	125	98.8 MHA	100	89.5	86.2	71.8	70	na
Manganese, Total	mg/L	--	na	5.5	6.2	6.07	6.24	5.33 MHA	9.62	5.17	4.29	4.06	3.76	na
Manganese, Dissolved	mg/L	--	na	na	6.6	5.97	5.87	5.21	5.08	4.91	4.60	4.2	4.04	na
Methane	ug/L	--	2400	2900	2800	11600	16200	4600 M7	4200	8530	16500	10600	7170	na
Nitrate as N (NO3-N)	mg/L	--	0.10 U	0.10 U	0.10 U	0.100 U M1	0.100 U	0.100 U M1	0.10 U	0.10 U	0.10 U	0.10 U	0.10 U	na
Nitrite as N (NO2-N)	mg/L	--	0.020 U	0.020 U	0.020 U	0.100 U	0.100 U	0.100 U	0.100 U	0.100 U	0.100 U	0.100 U	0.100 U	na
Nitrogen, Total Kjeldahl as N	mg/L	--	27	30	32	34.2 M1	43.1	50.4 M1	61.8	58.1	93.6	113 M1	130	na
Phosphate, Ortho as P	mg/L	--	0.027 B^	0.050 U	0.016 B	0.100 U	0.100 U	1.00 U RL1	0.100 U	0.100 U	0.100 U	0.100 U	0.100 U	na
Sulfate	mg/L	--	9.3	15	5.0 U	15.9		12.2 M7	88.7	140	76.3	81.9	165	na
Sulfide	mg/L	--	2.3	6.3	6.8	1	1.55 pH<12	2.00 U	2.00 U	2.00 U	2.00 U	2.00 U	2.00 U pH<12	na
Total Organic Carbon	mg/L	--	12	18	18	16.6 M1	7.42 ET	7.22 ET, M1	7.66 ET	16.9 ET	26.7 ET	25.4	26.7 ET	na
Benzene	ug/L	5	49	29	270	286	285	369 M1	551	554	786	1280	1580	na
Toluene	ug/L	2,000	3.7	1.5	10 U	21.6	18.6	22.8 M1	11.2 L1	5.15	10.7	26.7	14.4	na
Ethylbenzene	ug/L	700	91	57	500	715	536	585	789	671	890	831	1170	na
Xylenes	ug/L	10,000	75	29	220	734	232	279 M1	236 L1	556	235	277	117	na
Acenaphthene	ug/L	--	2.0 Ja	18	11	32.4	54.3	55.4	84.7	79.4	80.6	101	109	120 MHA
Acenaphthylene	ug/L	--	50	380	270	0.0850 U	0.0944 U	0.0850 U	0.0850 U	0.0870 U	0.0870 U	0.0870 U	1.74 U	0.870 U,MHA
Anthracene	ug/L	--	0.050 U	0.24 U	0.051 U	0.0113	0.0721 J	0.136 J	0.217	0.125 J	0.160 J	0.265	0.0100 U	0.192
Benzo(a)anthracene	ug/L	0.1	0.13 U	0.62 U	0.13 U	0.00558	0.00333 U	0.00300 U	0.01 J	0.00500 U	0.00500 U	0.00500 U	0.00500 U	0.00500 U
Benzo(a)pyrene	ug/L	0.2	0.13 U	0.62 U	0.13 U	0.0320 U	0.0356 U	0.0320 U	0.0320 U	0.00800 U	0.00800 U	0.00800 U	0.00800 U	0.00800 U
Benzo(b)fluoranthene	ug/L	0.2	0.050 Ua	0.24 U	0.051 U	0.0130 U	0.0144 U	0.0130 U	0.0130 U	0.0280 U	0.0280 U	0.0280 U	0.0280 U	0.0280 U
Benzo(g,h,i)perylene	ug/L	--	0.20 U	0.95 U	0.20 U	0.00900 U	0.0100 U	0.00900 U	0.00900 U	0.00800 U	0.00800 U	0.00800 U	0.00800 U	0.00800 U
Benzo(k)fluoranthene	ug/L	0.2	0.050 U	0.24 U	0.051 U	0.0150 U	0.0167 U	0.0150 U	0.0150 U	0.00700 U	0.00700 U	0.00700 U	0.00700 U	0.00700 U
Chrysene	ug/L	0.2	0.13 U	0.62 U	0.13 U	0.0338 J	0.00556 U	0.00500 U	0.00500 U	0.00800 U	0.00800 U	0.00800 U	0.00800 U	0.00800 U
Dibenzo(a,h)anthracene	ug/L	0.2	0.30 U	1.4 U	0.30 U*	0.0100 U	0.0111 U	0.0100 U	0.0100 U	0.0100 U	0.0100 U	0.0100 U	0.0100 U	0.0100 U
Fluoranthene	ug/L	--	0.13 U	0.22 Ja	0.13 U	0.0100 U	0.0111 U	0.0100 U	0.0100 U	0.0100 U	0.0100 U	0.198	0.0100 U	0.0100 U
Fluorene	ug/L	--	0.25 U	1.8	1.2	10.1	20.3	33.5	64.2	8.06	11.5	91.7	53	54 MHA
Indeno(1,2,3cd)pyrene	ug/L	0.4	0.13 U	0.62 U	0.13 U	0.00700 U	0.00778 U	0.007	0.00700 U	0.00600 U	0.00600 U	0.00600 U	0.00600 U	0.00600 U
Naphthalene	ug/L	100	56	520	360	167	727 B	719	506	211	324	703	R	429 MHA
Phenanthrene	ug/L	--	0.099 Ua	0.48 U	0.10 U	0.544	2.4	1.65	2.95	2.17	2.63	3.52	0.100 U	0.00500 U,M1
Pyrene	ug/L	--	0.25 U	1.2 U	0.25 U	0.0190 U	0.0211 U	0.0190 U	0.0248 J	0.0170 U	0.0170 U	0.0170 U	0.0170 U	0.0170 U
TPH as Gasoline	mg/L	--	na	na	na	na	na	na	na	na	na	na	8.53	na
Diesel	ug/L	--	na	na	na	na	na	na	na	na	na	na	5340 N1,Q	na
Gasoline	ug/L	--	na	na	na	na	na	na	na	na	na	na	13000 B,N1,Q	na
Motor Oil	ug/L	--	na	na	na	na	na	na	na	na	na	na	512 N1,Q	na
Total Extractable Hydrocarbons	ug/L	--	na	na	na	na	na	na	na	na	na	na	18900	na
BOD - 5 Day	mg/L	--	na	na	na	na	na	na	na	na	na	na	16.4	na
Chemical Oxygen Demand	mg/L	--	na	na	na	na	na	na	na	na	na	na	75.7 M1	na
Phosphorous, Total	mg/L	--	na	na	na	na	na	na	na	na	na	na	na	1.75

TABLE 1
GROUNDWATER ANALYTICAL RESULTS
MIDAMERICAN ENERGY COMPANY
PEOPLES NATURAL GAS SITE
DUBUQUE, IOWA

Notes:
-- = Remediation Goal not established.
BTOC = Below Top Of Casing
na-Not Analyzed
ns-Not Sampled
* = LCS, LCD, ELC, ELD, CV, MS, MSD, Surrogate: Batch QC exceeds the upper or lower control limits.
a-Concentration is below the reporting limit
B - Analyte was detected in the associated Method Blank
C9 - Calibration Verification recovery was outside the method control limits for this analyte. The LCS for this analyte met CCV acceptance criteria, and was used to validate the batch.
CIN - The % RSD for this compound was above 15%. The average % RSD for all compounds in the calibration met the 15% criteria specified in EPA methods 8260B/8270C
ET - Matrix interference in sample is causing an endpoint timeout.
FM - Elevated detection limits due to sample foaming.
H - Sample analysis performed past method-specified holding time.
J-Estimated concentration below the reporting limit
L1- laboratory Control Sample and/or Laboratory Control Sample Duplicate recovery was outside control limits.
L5 - Laboratory Control Sample was outside of acceptance Limits. The MS or MSD was used to validate the batch.
M1 - The MS and/or MSD were outside control limits.
MHA-Due to high levels of analyte in the sample, the MS/MSD calculation does not provide useful spike recovery information.
N1 - See case narrative.
pH<12 - Sample received at pH<12. It was adjusted correctly prior to analysis.
pH>2 - Sample received at pH>2. It was adjusted correctly prior to analysis.
P-HS = The sample container contained headspace.
Q - Poor chromatographic match to standard.
R - Sample result rejected; not useable.
S3 - Post digestion spike is out of acceptance limits for this analyte
U-Analyte not detected at or above reporting limit
ug/l - micrograms per liter
ZX - Due to sample matrix effects, the surrogate recovery was outside the control limits.



DESIGNED BY	KEVIN ARMSTRONG
DRAWN BY	NORA DAY
CHECKED BY	JANET BALDWIN
APPROVED BY	KEVIN ARMSTRONG
PROJECT MANAGER	KEVIN ARMSTRONG

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SCALE IN FEET

MANAGING OFFICE: DES MOINES, IOWA

PROJECT: MIDAMERICAN ENERGY COMPANY PEOPLES NATURAL GAS SITE DUBUQUE, IOWA

TITLE: PREDOMINANT GROUNDWATER FLOW DIRECTIONS IN SILTY SAND AQUIFER

MWH

FIGURE 1

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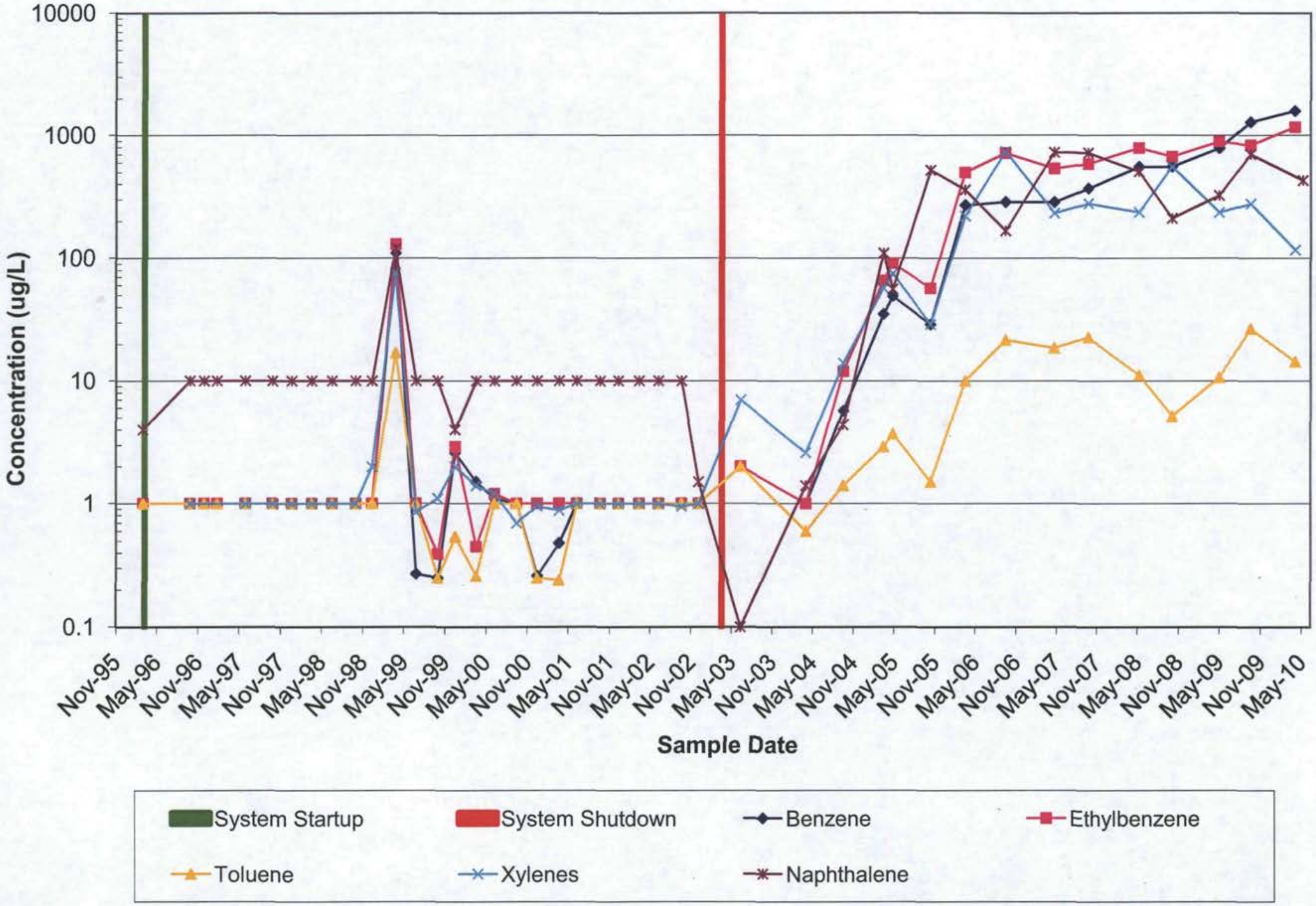


DESIGNED BY	MWH	MANAGING OFFICE	DES MOINES, IOWA
DRAWN BY	K. ARMSTRONG	PROJECT	MIDAMERICAN ENERGY COMPANY PEOPLES NATURAL GAS SITE DUBUQUE, IOWA
CHECKED BY	J. BALDWIN		
APPROVED BY	K. ARMSTRONG	TITLE	P-112 CONCENTRATIONS
PROJECT MANAGER	K. ARMSTRONG	FIGURE 2	REVISION 1
		FILE NAME	

MWH

ATTACHMENT A

BIO-TRAP®
ADVANCED DIAGNOSTIC SAMPLERS

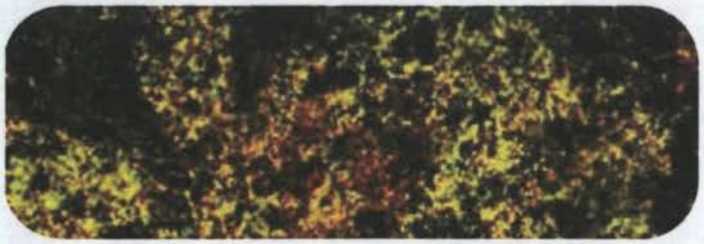
What types of samplers are available?

Bio-Trap samplers are available in a wide variety of configurations that can be tailored to answer your site-specific questions.

Standard: Basic Bio-Trap® Samplers in the simplest terms are a replacement for collecting groundwater samples using a conventional approach. Most microbes prefer to be attached to a surface rather than free floating and this passive sampler provides a large surface area for the microbes to colonize. Results generated using this approach have been shown to minimize the variability associated with traditional sampling approaches. Bio-Traps biofilms have also been shown to directly reflect spatial and temporal changes in aquifer microbial community structure plume which could not be determined from groundwater analysis. Standard Bio-Trap® Samplers are primarily used during site characterization and routine monitoring activities to:

- Quantify specific microbes or contaminant degrading bacteria (e.g. *Dehalococcoides* spp.)
- Evaluate monitored natural attenuation (MNA)
- Compare microbial populations from different sampling points
- Monitor shifts within microbial communities following biostimulation

Standard Bio-Trap® Samplers are designed for microbial analyses using a variety of molecular biological tools but can also be configured for some chemical and geochemical analyses.



microbialinsights
www.microbe.com

2340 Stock Creek Blvd.
Rockford, TN 37853-3044
Phone: 865.573.8188

Baited: As the name suggests, Bio-Trap® Samplers can be "baited" with various amendments or compounds to answer site-specific questions. In the past, project managers have been forced to turn to laboratory microcosms or small-scale pilot studies to evaluate bioremediation as a treatment alternative. While microcosm experiments with native site materials can show biodegradation in the laboratory, duplication of *in situ* conditions is difficult and the results may not extrapolate to the field. Pilot studies are performed on site but are often prohibitively expensive as an investigative tool. Baited Bio-Trap® Samplers are designed to create discrete in situ microcosms that can be used to:

- Evaluate monitored natural attenuation versus enhanced bioremediation
- Compare effectiveness of different amendments (e.g. HRC®, EOS®, sodium lactate, molasses, etc.) designed to stimulate bioremediation
- Prove that biodegradation is occurring (^{13}C-labeled compounds - Stable Isotope Probing)
- Estimate relative rates of degradation for a specific contaminant (i.e. MTBE, TBA etc.)
- Address specific questions such as:
  - Is benzene being degraded at my site?
  - Will sulfate amendments stimulate bioremediation?
  - Will sodium lactate increase the concentration of known dechlorinating bacteria?

Baited Bio-Trap® Samplers can be amended with a number of compounds including:

- Sodium acetate
- Sodium lactate
- Potassium lactate
- HRC®
- Molasses
- Vegetable oil
- EOS®
- Sodium phosphate
- Sulfate
- Nitrate
- Ammonium chloride
- Elemental sulfur
- Calcium carbonate
- Iron (III)
- ^{13}C-labeled contaminants
  - Benzene
  - Toluene
  - Xylene
  - MTBE
  - TBA
  - Chlorobenzene
  - TCE
  - DCE
  - VC
- Fluorinated surrogates for tracing chlorinated compounds
  - TCE
  - DCE
- And more!

mi BIO-TRAP®

ADVANCED DIAGNOSTIC SAMPLERS

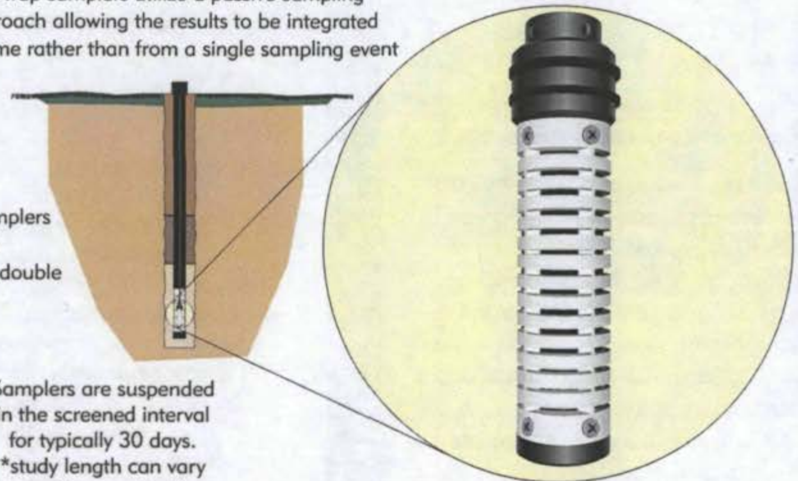
Catch Remediation in the Act... ***Trap It!***

What are Bio-Trap® Samplers?

Bio-Trap® Samplers are passive sampling tools that collect microbes over time for the purpose of better understanding biodegradation potential. The key to the Bio-Trap® approach is a unique sampling matrix, Bio-Sep® beads. The beads are 2–3 mm in diameter and are engineered from a composite of Nomex® and powdered activated carbon (PAC). When a Bio-Trap® Sampler is deployed in a monitoring well, the Bio-Sep® beads adsorb contaminants and nutrients present in the aquifer essentially becoming an *in situ* microcosm with an incredibly large surface area (~600 m^2/g) which is colonized by subsurface microorganisms. Once recovered from a monitoring well (30-60 days after deployment), DNA, RNA, or PLFA can be extracted from the beads for CENSUS® or PLFA assays to evaluate the microbial community.

A modern approach to microbial sampling

Bio-Trap samplers utilize a passive sampling approach allowing the results to be integrated over time rather than from a single sampling event



Multiple Bio-Trap samplers can be isolated from one another using a double seal cap assembly

Samplers are suspended in the screened interval for typically 30 days. *study length can vary depending on objectives

Sampling Matrix: Bio-Sep® Beads

Exterior of Bio-Sep bead

A key to this sampling approach is the use of Bio-Sep® beads as the sampling matrix. The unique properties of these beads allow them to mimic environmental conditions very well.

Interior of Bio-Sep bead

Bio-Sep® beads provide a large surface area within the bead for microbial attachment. Most microbes prefer to be attached to a surface rather than be free floating.

Lactate amended Bio-Sep® bead

Fishin' for microbes! "Baited" Bio-Trap® samplers can be used to evaluate the microbial response to a wide range of amendments (electron donors and acceptors, etc.).

*see reverse for more details

Samplers can be analyzed using a wide variety of analyses including:

Molecular Biological Tools
- CENSUS® (qPCR)
- PLFA
- DGGE
- SIP

Chemical Analysis
Geochemical Parameters
And more!

microbialinsights

www.microbe.com

ATTACHMENT B

YOUR NATURAL SOLUTIONS

Patented Methods for *In Situ* Bioremediation

Product Information Sheet

EAS™

Electron Acceptor Solution

Description & Use:	Sulfate reduction and methanogenesis appear to be the dominant natural degradation processes at most sites (Wiedemeier et al., 1999). A BP – EPA study on the median consumptions of electron acceptors at 74 sites concluded that most hydrocarbon plumes are anaerobic and depleted of sulfate. Based on a solid body of published scientific evidence, adding electron acceptors (**EAS™**, U.S. Patent # 7,138,060) to groundwater will aid in increased degradation. The addition of **EAS™** will stimulate biodegradation by providing a **soluble**, readily available electron acceptor. In the presence of elevated SO_4^{-2}, anaerobic groundwater bacteria use the petroleum-hydrocarbons for carbon and energy while mineralizing the hydrocarbons to CO_2 and H_2O. In addition, SO_4^{-2} reduction consumes protons increasing the pH and enhancing methanogenesis. The EAS Technology simply enhances the environmental conditions that exist within a contaminant plume by replenishing a natural groundwater compound that the bacteria require to degrade the contaminants In order to evaluate your site-specific conditions, EOS Remediation suggests site-specific evaluation of the groundwater geochemistry to ensure **EAS™** application success is maximized. Upon request, a protocol with suggested sampling approaches and analyses is available for interested parties' review.
Applications	**Injecting EAS™** – Inject via injection point or through conventional drilling or Geoprobe® rod under zero to low pressure. The screen should bracket the water table with 3-4 feet above the water table and 1-2 feet below the water table. **EAS™** can be injected through existing piping in place (SVE, pump and treat, infiltration galleries, etc). No chase water is needed and the amount of **EAS™** required will be relatively low.
Packaging:	**EAS™** is shipped in 55-gallon drums, 275-gallons totes or tanker truck.
Storage & Handling:	Store **EAS™** indoors and protect from exposure to temperature extremes (<32ºF or > 120º F). **EAS™ will freeze at –32 F. Freezing does not affect product quality.** Workers should use eye protection and prevent skin contact. Consult the MSDS for additional information before using **EAS™.** Clean up spilled product promptly and dispose of in accordance with all regulations. For best performance, use within 180 days of delivery.

EOS Remediation, LLC

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MATERIAL SAFETY DATA SHEET

EAS™, Electron Acceptor Solution

D.O.T. HAZARD CLASSIFICATION: NONE

MANUFACTURER'S NAME
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DATE OF PREPARATION
8/22/2008

SECTION I - PRODUCT IDENTIFICATION

PRODUCT NAME: **EAS™, Electron Acceptor Solution**
Product Description: **Magnesium sulfate, water solution**
CAS NUMBER: 7487-88-9

SECTION II - COMPOSITION

Chemical Name	CAS#	Wt%	OSHA PEL	ACGIH TLV
Sulfuric acid, magnesium salt; Magnesium sulfate	7487-88-9	5 – 27%	Not Established	Not Established
Water	7732-18-5	Balance	Not Established	Not Established

SECTION III - HAZARDS IDENTIFICATION

Emergency Overview: Colorless, transparent, odorless liquid. Noncombustible. At very high temperatures, magnesium oxide, sulfur dioxide, and sulfur trioxide may be generated. May cause mild eye irritation.

Eye contact: May cause mild irritation to the eyes.
Skin contact: No known adverse effects.
Inhalation: Causes nausea, vomiting, abdominal cramps, and diarrhea.
Ingestion: Causes nausea, vomiting, abdominal cramps, and diarrhea.
Chronic hazards: No known chronic hazards. Not listed by NTP, IARC or OSHA as a carcinogen.
Physical hazards: Spilled material can be slippery.

SECTION IV - FIRST AID MEASURES

Eye: In case of contact, immediately flush eyes out with plenty of water for at least 15 minutes. Get medical attention if irritation persists.
Skin: Not applicable.
Inhalation: Remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention.
Ingestion: If large quantities of this material are swallowed, call a physician immediately. Do NOT induce vomiting unless directed to do so by a physician. Never give anything by mouth to an unconscious person.

SECTION V - FIRE AND EXPLOSION HAZARD DATA

Flammable limits:	This material is noncombustible.
Extinguishing Media:	This material is compatible with all extinguishing media
Hazards to fire-fighters:	See Section III for information on hazards when this material is present in the area of a fire.
Fire-fighting equipment:	The following protective equipment for fire fighters is recommended when this material is present in the area of a fire: chemical goggles, body-covering protective clothing, self-contained breathing apparatus.

SECTION VI - ACCIDENTAL RELEASES

Personal protection:	Wear chemical goggles, See section VIII
Environmental Hazards:	Sinks and mixes with water. No adverse effects known. Not a listed toxic chemical under SARA Title III, §313 40 CFR Part 372. Not a CERCLA Hazardous Substance under 40 CFR Part 302.
Small spill cleanup:	Mop up discharged material. Flush residue with water. Observe environmental regulations.
Large spill cleanup:	Keep unnecessary people away; isolate hazard area and deny entry. Do not touch or walk through spilled material. Stop leak if you can do so without risk. Prevent runoff from entering into storm sewers and ditches which lead to natural waterways. Isolate, dike and store discharged material, if possible. Use sand or earth to contain spilled material. If containment is impossible, flush with large quantities of water.
CERCLA RQ:	There is no CERCLA Reportable Quantity for this material.

SECTION VII - HANDLING & STORAGE

Handling:	Avoid breathing mist. Promptly clean up pills.
Storage:	Keep containers closed. Recommended storage temperature 50°-120°F.

SECTION VIII - EXPOSURE CONTROLS / PERSONAL PROTECTION

Engineering controls:	Use with adequate ventilation. Safety shower and eyewash fountain should be within direct access.
Respiratory protection:	Use a NIOSH-approved dust and mist respirator where mist occurs. Observe OSHA regulations for respirator use (29 C.F.R. §1910.134)
Skin protection:	Wear gloves if irritation occurs.
Eye protection:	Wear chemical goggles.

SECTION IX - PHYSICAL & CHEMICAL PROPERTIES

Appearance:	Transparent liquid.
Color:	Colorless.
Odor:	Odorless.
pH:	Approximately 8.5
Specific gravity:	1.22 g/cm3 at 20° C for a 20% solution
Solubility in water:	Miscible.

SECTION X - STABILITY & REACTIVITY

Stability: This material is stable under all conditions of use and storage.
Conditions to avoid: None.
Materials to avoid: Metal hydrides and other water reactive materials.
Hazardous decomposition products: At very high temperatures, magnesium oxide, sulfur dioxide, and sulfur trioxide may be generated.

SECTION XI - TOXICOLOGICAL INFORMATION

Acute Data: When tested for primary irritation potential, a similar material caused mild eye irritation.

RTECS reports the following data for magnesium sulfate:
Oral TDLo= 428 mg/kg in man 351 mg/kg in women

SECTION XII - ECOLOGICAL INFORMATION

Eco toxicity: Data not available.
Environmental Fate: This material is not persistent in aquatic systems and does not contribute to BOD. It does not bioconcentrate up the food chain.
Physical/Chemical: Sinks and mixes with water.

SECTION XIII - DISPOSAL CONSIDERATIONS

Classification: Disposed material is not a RCRA hazardous waste.
Disposal Method: Dispose in accordance with local, state, and federal regulations.

SECTION XIV - TRANSPORTATION INFORMATION

DOT UN Status: This material is not regulated hazardous material for transportation.

SECTION XV - REGULATORY INFORMATION

CERCLA: No CERCLA Reportable Quantity has been established for this material.
SARA TITLE III: Not an Extremely Hazardous Substance under §302. Not a Toxic Chemical under §313. Hazard Categories under §§311/312: Acute
TSCA: All ingredients of this material are listed on the TSCA inventory.

SECTION XVI - OTHER INFORMATION

The information contained herein is based on available data and is believed to be correct. However, EOS Remediation, LLC. makes no warranty, expressed or implied, regarding the accuracy of this data or the results to be obtained thereof. This information and product are furnished on the condition that the person receiving them shall make his/her own determination as to the suitability of the product for his/her particular purpose.

EAS™ Protocol (Electron Acceptor Solution)

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This EAS Protocol is intended to describe site qualifications, application methods, guidelines for analysis of geochemical indicators and a brief technical overview of EAS™.

INTRODUCTION:

EAS™ is a cost effective, safe and easy method for enhancing natural degradation of BTEX, MTBE, petroleum hydrocarbons and other constituents. The EAS™ technique enhances natural processes that are already occurring, substantially increasing the degradation rate and potentially decreasing time of remediation. This process is effective where oxygen has been depleted in the groundwater. It does not induce anaerobic degradation if it is not already established at a site naturally. It is intended for dissolved phase and residual contamination at or below the water table. It is best applied at or near the contaminant source area. It is not intended for treatment of free phase contamination or for soil vapor.

QUALIFYING A SITE FOR EAS

Data for evaluating all of the above criteria should be available from a standard site assessment, plus additional groundwater analyses of selected background wells (one or more upgradient and two side gradient) and plume wells (preferably in the source zone and further out in the dissolved plume).

The following criteria are recommended to qualify a site for EAS™ addition to enhance natural attenuation:

1. The dissolved phase plume should be well defined with at least one upgradient and two side gradient monitoring points outside the plume (to define background concentrations of electron acceptors), and enough monitoring points side- and downgradient to define the edge of the dissolved phase plume.

2. There should be enough data to define the probable source area. Contour maps of contaminant concentration are helpful. Ideally the process for defining the source area should include site history (e.g. location of USTs, lines, etc.) as well as analytical data.

3. The dissolved phase plume should be stable or declining.

4. Sulfate should be present naturally in background groundwater, and should show a clear inverse relationship with dissolved hydrocarbons in the groundwater. In other words, sulfate levels should be at background levels outside and upgradient of the plume and sulfate levels should be depleted in the heart of the plume where hydrocarbons are present, thereby indicating sulfate reduction is already occurring.

5. There should be clear indication that the heart of the plume is already undergoing natural anaerobic degradation. This may be accomplished using at least two simple geochemical parameters, one of which must be the sulfate (described above). Geochemical indicator parameters may be depletion in the plume of DO, or nitrate, or sulfate; and/or increases in the plume of dissolved iron, or dissolved CO_2, or dissolved methane, or dissolved hydrogen; and/or ORP in negative millivolts in the heart of the plume. Note that "depletion" does not mean a zero or non-detect value, but simply a substantial reduction in concentration in the plume as compared to the background concentration. If desired (optional not mandatory), an rRNA bacterial CENSUS analysis or a phospholipid fatty acid (PLFA) analysis may be run from soil or groundwater from the heart of the plume to determine presence and relative abundance of bacterial groups such as iron reducing, nitrate reducing, sulfate reducing, etc.

6. Hydraulic conductivity in the treatment zone should be 10^{-4} cm/s (0.3 ft/d) or greater. This will allow for adequate dispersion of EAS™.

7. Ideally, dissolved iron in the heart of the plume (at or near the source area) should be 2 mg/l or greater. One sample is sufficient for this because iron concentrations will vary substantially away from the source area. If an iron analysis is not 2 mg/l or greater, then groundwater in the source area should be analyzed for other metals such as manganese, arsenic, lead, zinc, etc. This indicates there will be reduced metals available to combine (mineralize) with generated sulfide to prevent or mitigate generation of H_2S. The end result will be that both sulfide and dissolved metals are removed from groundwater.

8. The average depth to the water table should be 3 feet or more (some tolerance may be given here on a site specific basis). This is primarily to ensure that the sulfide generated in the microbial reaction has sufficient residence time and contact area to either precipitate as metal sulfide (based on metal availability) or get oxidized to sulfate in the vadose zone.

RECOMMENDED EAS™ APPLICATION **METHODS**

EAS™ may be added to the source zone and dissolved phase plume through several different means:

- EAS™ may be added to wells or trenches that penetrate the water table. (This method provides faster results.) If injected through an injection well, the screen should bracket the water table.

- EAS™ may be applied to unpaved permeable surface areas (such as grass or gravel areas) for infiltration to the water table. Time must then be allowed for infiltration of solution to the water table.

- EAS™ may be added to excavation backfills. It is usually mixed with other backfill at no greater than about 5% by weight to avoid compaction concerns. Time must then be allowed for dissolution of EAS™ by rain water percolation.

Where to apply

The type of application and area to be applied will be site specific. It is recommended that EAS™ applications focus on the contaminant source area, with the resulting reactions zone being the source area and dissolved plume area downgradient of the source. The thickness of the reaction zone will be the saturated screened interval of the application wells or trench, or if the applications are made above the water table (such as surface applications) the reaction zone will be the upper few feet of groundwater below the water table.

How much EAS™

Before application, the amount of sulfate required should be calculated based on the estimated mass of hydrocarbon contaminants in the source area, and the utilization and safety factors described in the Appendix. Stoichiometrically, it takes approximately 4.7 grams of sulfate to degrade one gram of BTEX. There are usually other soluble hydrocarbons or chemical sinks in the plume area; therefore the amount of sulfate is increased by a multiplication safety factor (usually 2).

There are various means to calculate contaminant mass in the source area. EOS Remediation has developed an EAS™ Design Tool. Please fill out the EAS™ Site Evaluation Form (www.eosremedaition.com) and submit to tparker@eosremediation.com. The EOS Remediation staff will calculate the suggested recommended amount of EAS™ at your site.

Typically 3-4 application will be needed. The number of applications is going to be dependant on performance monitoring, application technique, groundwater velocity and remediation objectives. Some sites have been remediated with only one EAS™ injection.

MONITORING NATURAL ATTENUATION AND ANAEROBIC ZONES

The three primary lines of evidence to show that natural attenuation is occurring and that anaerobic zones are developing are the following:

1. Geochemical indicators (preferred method: should meet two or more criteria)
2. Stable or decreasing plume over time
3. Documented presence of hydrocarbon degrading organisms (optional)

Observing simple groundwater geochemical parameters, such as the change in concentration of electron acceptors (such as oxygen or sulfate) across a plume, can provide definitive evidence of microbial degradation of contaminants, and provide quantitative data regarding rates of degradation and the contribution of different processes.

A stable or decreasing plume over time does indicate attenuation is occurring, but does not prove that biodegradation is a major component or whether the degradation is aerobic or anaerobic.

Many microbial analyses can be time consuming or expensive. Sometimes the results can be misleading. For example, plate counts frequently detect less than one percent of the microbial species present. Phospholipid fatty acid analysis (PLFA) does an excellent job of identifying the relative abundance of bacterial groups such as aerobic, iron reducing and sulfate reducing bacteria, but is not effective in identifying archaea or other nonbacterial microbes. For these reasons, the use of geochemical indicators is preferred with optional use of microbial analyses only if needed for confirmation.

These parameters should be viewed in relation to background and in relation to the plume geometry. For example, if microbial degradation zones are active, oxygen, nitrate and sulfate will decrease in concentration from upgradient background, to minimal concentrations in their respective terminal electron acceptor process (TEAP) zones. Dissolved iron will increase from near non-detect in background to substantial concentrations (close to 5 mg/l or more) in and downgradient of the iron reducing zone. Carbon dioxide and dissolved hydrogen will also increase in the plume as groundwater passes through the TEAP zones. Figure 1 gives a flow chart of different electron acceptor concentrations and their corresponding TEAP zones.

(It should be noted that field measurements of dissolved oxygen are often erroneous, and they should be viewed with care. This is because old instruments that measure oxygen concentration using voltage potential are difficult to keep in calibration, their membranes go bad, and samples may be mishandled at the surface allowing exposure to air and oxygen. Use of newer optical instruments and low flow methods are strongly advised.)

Required and Optional Groundwater Analyses:

	Required	**Field**	**Lab**
Tier 1	DO, pH, Redox, Temp	Field Equipment	
	Dissolved Iron		(mod. 7199)
	Total Iron		(mod. 7199)
	Nitrate/nitrite		(method 9056 or equivalent)
	Sulfate		(method 9056 or equivalent)
	Sulfide		method 376.1 or equivalent
	Alkalinity		
(Note: Kits are available for field analyses of some inorganic ions such as iron, sulfate and nitrate, etc. If the detection limits are sufficient, these may be adequate to determine trends between background and plume concentrations, otherwise laboratory analyses will be necessary.)			

	Optional	
	Inorganics	**Dissolved Gasses**
Tier 2	Ca, Mg, Na, K	CO2
	Chloride	H2
	Carbonate	Methane
	Bicarbonate	H2S
	Bromide	
	TOC	

	Optional	Notes
Tier 3	**Microbial Analysis**	
	qPCR 1st Target IRB/SRB (Iron-sulfate reducing bacteria)	Estimates the abundance of sulfate and iron reducing by targeting bacteria within the deltaproteobacteria group
	Additional qPCR Target DSR (Dissimilatory Sulfite Reductase)	Gene found within sulfate-reducing bacteria
	Additional qPCR Target BssA (Benzyl Succinate Synthase)	BssA mediates the activation of toluene through addition of fumarate to the methyl group
	Biotraps	These are a passive sampling tool for evaluating microbiological and certain redox conditons in-situ. They can be baited with a variety of amendments, such as sulfate substrate, so as to evaluate remediation options.
	Other	
	34S Isotope analysis	It's likely that dissolved hydrogen analysis will provide the same information at a lesser cost. This could be utilized as an additional line of evidence of degradation activity, if needed.
	Soil Analysis/Acid Volatile Sulfides	If accumulation of ferrous sulfides in soil from the sulfate remediation is a concern the analysis of AVS from soils taken from the saturated zone could help determine if this is occurring. The drawback is additional drilling would be necessary.

INTREPRETING GEOCHEMICAL INDICATORS

Evidence of Anaerobic Degradation

Lateral changes in the concentration of some inorganic ions in groundwater across a hydrocarbon plume may provide indicators that microbial degradation is occurring and whether it is aerobic or anaerobic (Chapelle, 2001).

For example, if oxygen is abundant in background-groundwater and it is essentially depleted in the dissolved phase plume area, then we can assume that aerobic degradation has occurred. Similarly, if sulfate is present in the background and depleted in the plume area, we can assume that sulfate reduction has occurred, which is anaerobic. Both systems may be active, but in different zones or areas of the plume. In most cases where groundwater is contaminated with petroleum hydrocarbons, oxygen is consumed on the upgradient side of the contaminant source area. The plume then undergoes anaerobic degradation in a hierarchy of successive zones depending on the availability of electron acceptors. Table 1 shows this hierarchy.

TEAP Zone Processes and Parameters				
Electron Acceptor	Reaction	Metabolic Byproduct	Utilization Factor for BTEX	Thermodynamic Reaction Preference
Oxygen	Aerobic	CO_2	3.14	Most Preferred
Nitrate	Anaerobic	N_2, CO_2	4.9	↓
Fe III (solid)	Anaerobic	Fe II*, CO_2	21.8*	↓
Sulfate	Anaerobic	H_2S, CO_2	4.7	↓
CO_2	Anaerobic	Methane*	0.78*	Least Preferred

Table 1: Hierarchy of terminal electron acceptor process (TEAP) zones and their associated processes and parameters. Utilization factors indicate how many grams of an electron acceptor are required to degrade one gram of BTEX (those with a * use the metabolite rather than the electron acceptor). They are from Wiedemeier, et al., 1999.

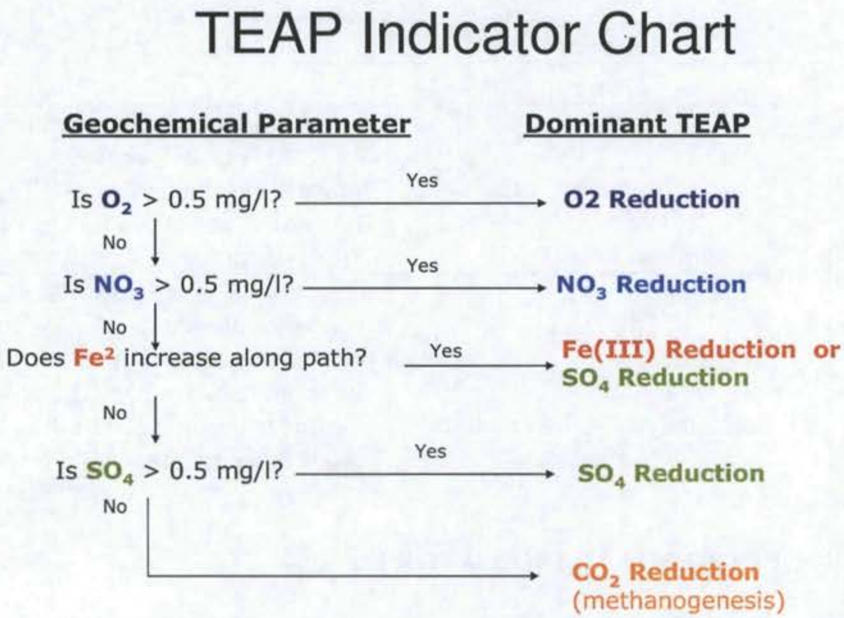

Figure 1: Simple geochemical parameters that define degradation zones. Chapelle, 2001.

Oxidation-reduction potential (ORP) is another good indicator of active TEAP zones and of which zone is dominant at a given monitoring well location. The ORP in a highly oxygenated background area will tend to be +200 millivolts or higher. Each successive TEAP zone in the hierarchy is more reducing, and the ORP decreases progressively into the negative millivolts range as groundwater migrates through the zones (Table 2).

TEAP Zone	ORP
Aerobic (O_2)	+200 millivolts or higher
O2 ➔ Nitrate & Mn^4 reduction	+200 ➔ +100 millivolts
Nitrate & Mn^4 ➔ Fe^3 reduction	+100 ➔ 0 millivolts
Fe3 ➔ SO^4 reduction	0 ➔ -100 millivolts
SO^4 ➔ Methanogenesis	-100 ➔ -200 millivolts
Methanogenesis	< -200 millivolts

Table 2: TEAP zones and oxidation reduction potential (ORP). Source: ESTCP Edible Oils Protocol

The typical distribution of these TEAP zones in a petroleum contaminated aquifer is shown in Figure 4. The relative size or area of a given process is usually dependent on the availability of a given terminal electron acceptor. Oxygen is usually only available in near surface groundwater in concentrations up to 7 or 8 mg/l. Nitrate is seldom available in quantity, and iron III (ferric iron) is nearly insoluble in water and is taken up by iron reducing bacteria from bio-available iron in soil minerals. In the iron reduction process, iron III is reduced to soluble iron II (ferrous iron) which is the primary cause for increased dissolved iron in the contaminated zone. This iron stays in solution until it either mineralizes with available sulfide from the sulfate reducing zone, or precipitates when redox conditions change in the aquifer downgradient of the plume.

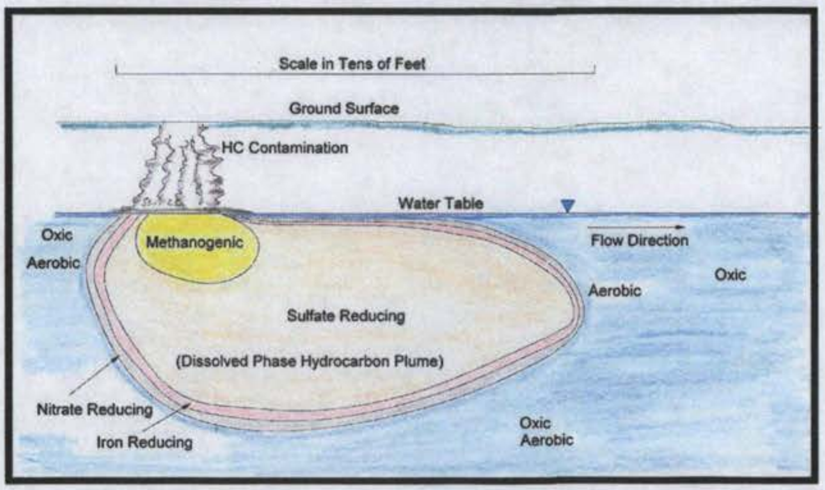

Figure 2: Typical profile of TEAP zones at a petroleum contaminated aquifer with a contaminant source area.

(This text was modified and excerpted from: Bruce et al., 2007, Anaerobic Degradation of Benzene was Enhanced through Sulfate Addition Substantially Increasing the HC Degradation Rate at a Central Indiana Site, in the Proceedings from the NGWA Hydrocarbons Conference, November 5&6 2007.)

SAFETY

There are safety considerations regarding the secondary MCL for sulfate in groundwater, and the potential for generating H_2S. Applications should be carefully planned regarding the reaction zone area (downgradient of application points) and the location and timing of application events. To facilitate monitoring and control, it is recommended that sulfate be applied in stages rather than one event.

Dissolved sulfate in the hydrocarbon plume is designed to be consumed in the degradation process. Therefore, if proper amounts are added, excess sulfate at down gradient receptors/compliance point should not exceed the secondary MCL of 250 mg/l or background sulfate concentration if higher than secondary MCL. Sulfate concentrations will temporarily be higher in the reaction zone of the plume but should not exceed the secondary MCL at the compliance point. Therefore plume wells and downgradient wells should be monitored for sulfate concentration and the applications adjusted accordingly.

Hydrogen sulfide (H_2S) has not been detected in soil gas to date at sites where sulfate has been applied (100+ sites as of mid 2009). However, if it is applied in close proximity to a residence or occupied dwelling, monitoring provisions should be made. If H_2S gas is detected, sulfate application should cease and appropriate measures taken to assure safety in nearby structures.

References

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NOTICE